



## **UGent\_Belgium**

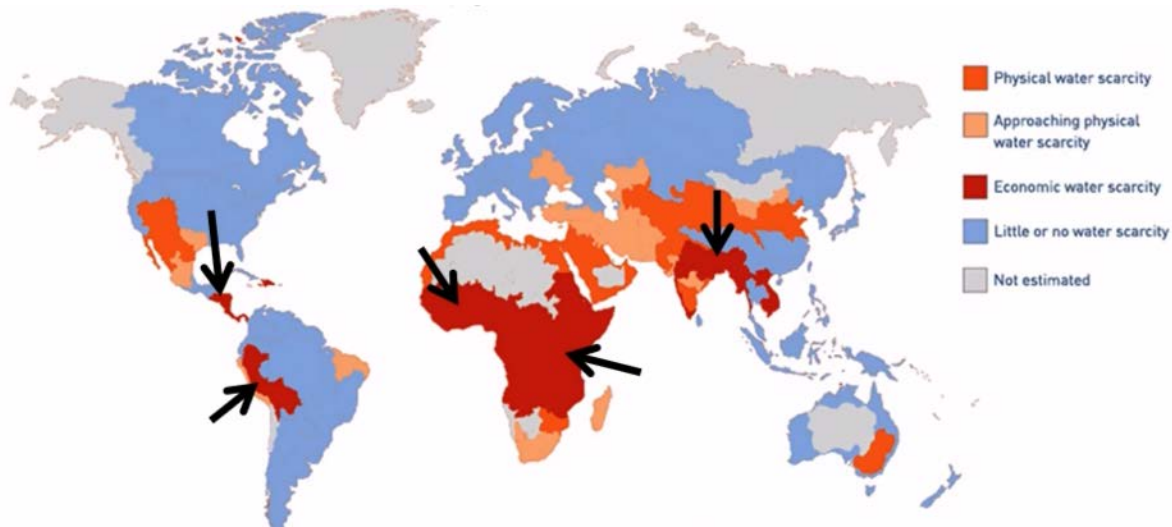
**PI's: Wim Van Criekinge, Marjan De Mey, Yves Briers**

**Sofie Lodens, Michiel Stock, Sandra Steyaert, Steven De Blicck,  
Bob Van Hove, Bram De Jaegher, David Bouwens  
Maarten Van Brempt, Bram Danneels, Griet De Clercq,  
Wouter Steyaert, Chari Vandenbussche**

# Introduction

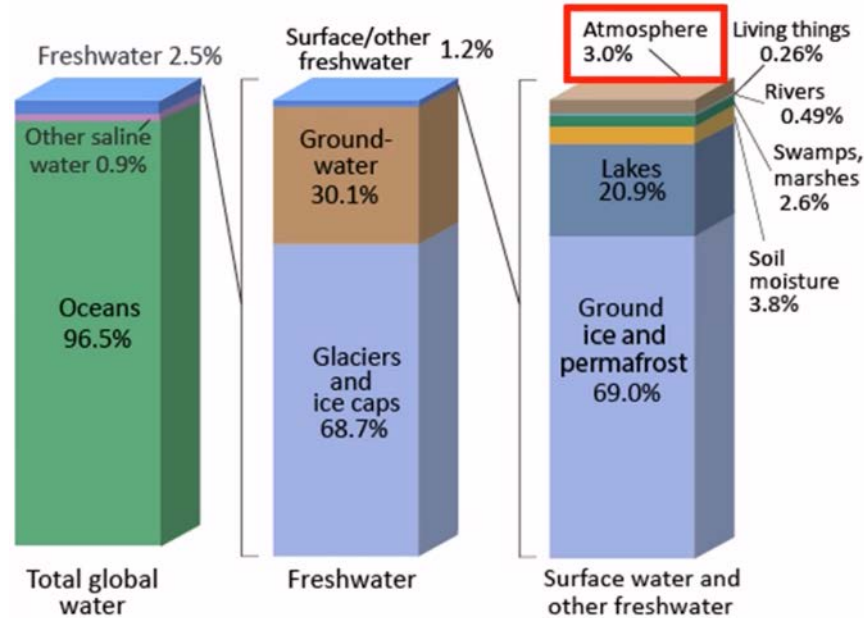
Tackle a global problem

## Water scarcity



# Introduction

Where to get more fresh water?

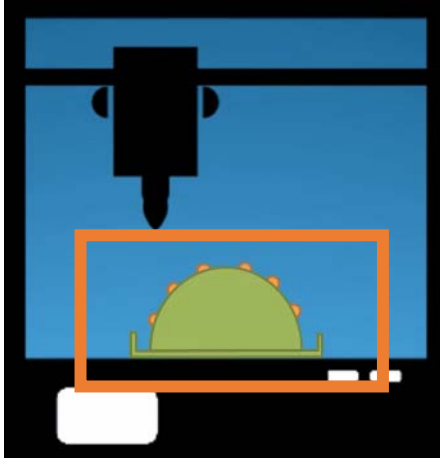


# Strategy

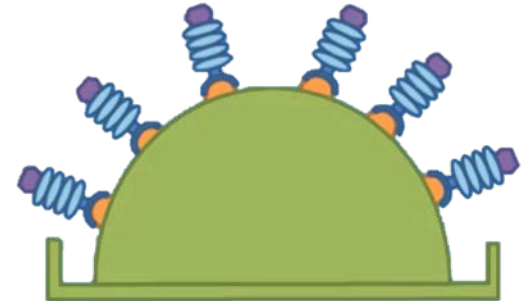
How to harvest atmospheric water?



Create 3D-printed optimized shape for water condensation and collection



Coat with biological nucleation proteins to improve water condensation



# Strategy

3D-printed optimized shape for water condensation and collection

Design structure based on fogstand beetle



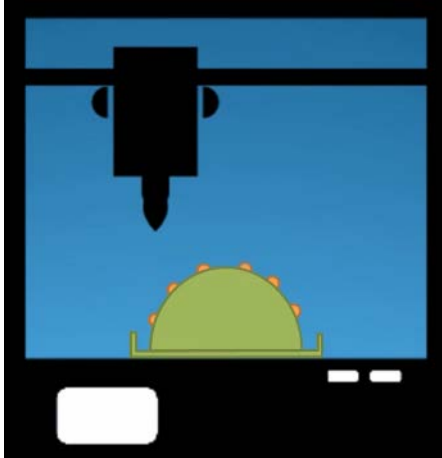
Fogstand beetle (*Stenocara gaeilipes*)

# Strategy

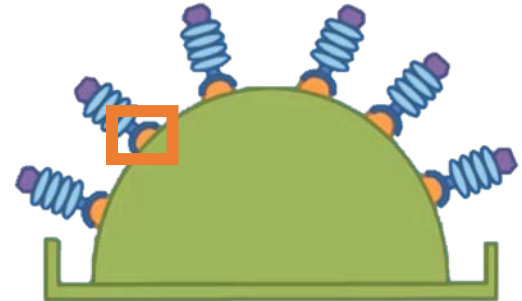
How to harvest atmospheric water?



Create 3D-printed optimized shape for water condensation and collection

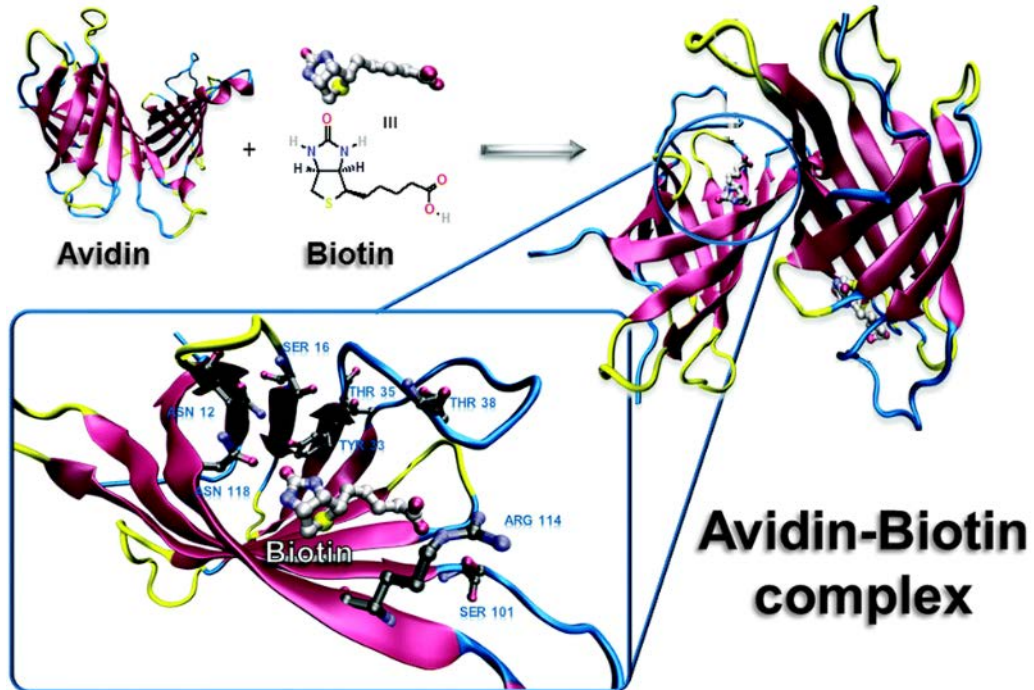


Coat with biological nucleation proteins to improve water condensation



# Strategy

How to coat a protein on a printed structure?



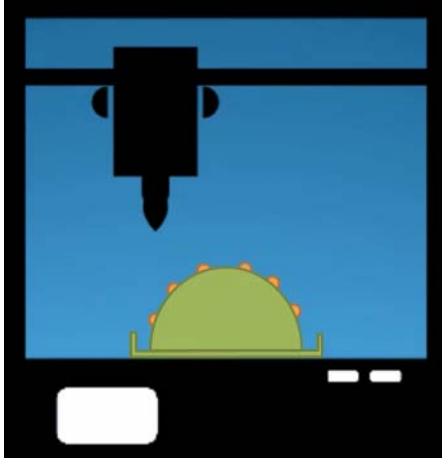
- PLA + biotine
- Fusion protein with streptavidin

# Strategy

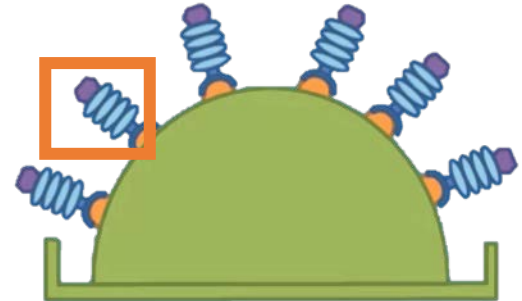
How to harvest atmospheric water?



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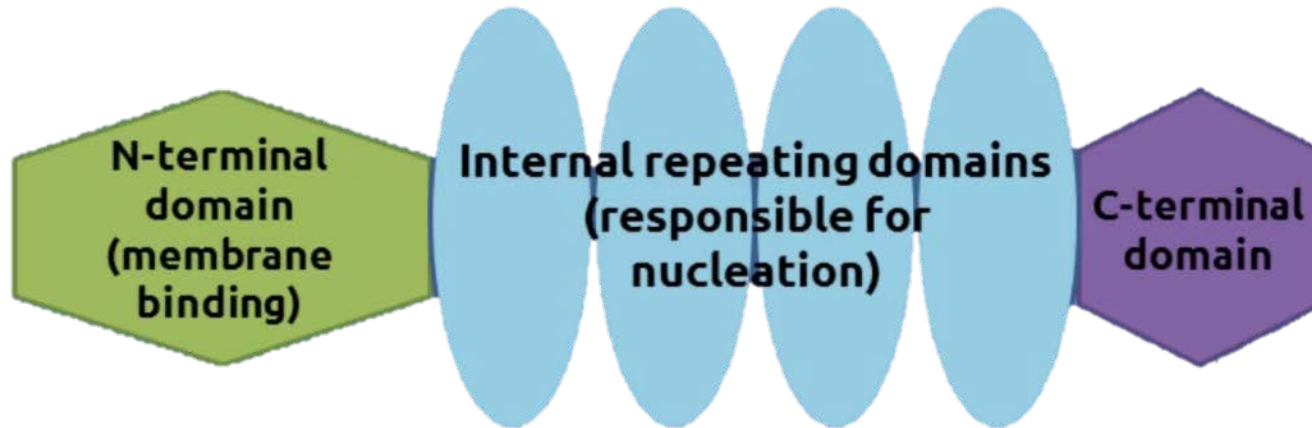




# Strategy

Condensation promoting protein?

... meet the *Pseudomonas syringae* **Ice Nucleating Protein (INP)**



# Strategy

Condensation promoting protein?



... meet the *Pseudomonas syringae* Ice Nucleating Protein (INP)

Microbial Showers...

## The Rain-Making Bacteria



Jay Hardy, CLS, SM (ASCP)

Jay Hardy is the founder and CEO of Hardy Diagnostics. He began his career in microbiology as a Medical Technologist in Santa Barbara, California.

In 1989, he began manufacturing culture media for the local hospitals. Today, Hardy Diagnostics is the third largest media manufacturer in the U.S.

During a cloudburst we often say that it's raining "cats and dogs". In reality, it's actually raining bacteria; a natural phenomenon that presents no cause for alarm. The sky, once thought of as a sterile void, is actually teeming with bacteria, which are vital for watering the plants below.

Biological precipitation, or the "bio-precipitation" cycle as it is called, starts when bacteria form colonies on the surface of plants. Winds will then sweep the bacteria into the atmosphere, and ice crystals form around them. Water molecules clump onto the crystals, making them bigger and bigger. The ice crystals turn into rain or snow and fall to the ground. When precipitation occurs, the bacteria have the opportunity



Nuclei are the seeds around which ice is formed. Snow and most rain begin with the formation of ice in clouds. Dust and soot can also serve as ice nuclei. But biological ice nuclei are different from dust and soot nuclei because only these biological nuclei can cause freezing at warmer temperatures.



Recently *Pseudomonas syringae* was also found in clouds, where it might help the formation of rain by acting as a condensation nucleus

## The bacteria that make it rain

POSTED BY SEDEER IN BACTERIA, MICROBIOLOGY, PLANTS

≈ 12 COMMENTS

Strange as it may seem, water doesn't actually freeze at zero degrees. In fact, even at temperatures as cold as  $-10^{\circ}\text{C}$ , water still needs help turning into ice.

Living creatures of all stripes have learned to take advantage of this curious fact in



# Work packages

WP1.  
Shape

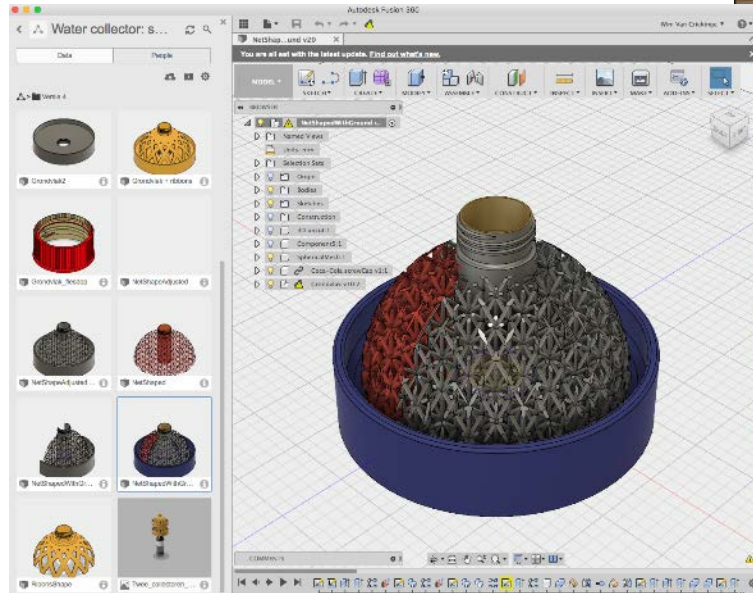
WP2.  
Filament

WP3.  
BioFunction

WP4.  
Functional Assay



# WP 1: Shape



# WP 2: Filament

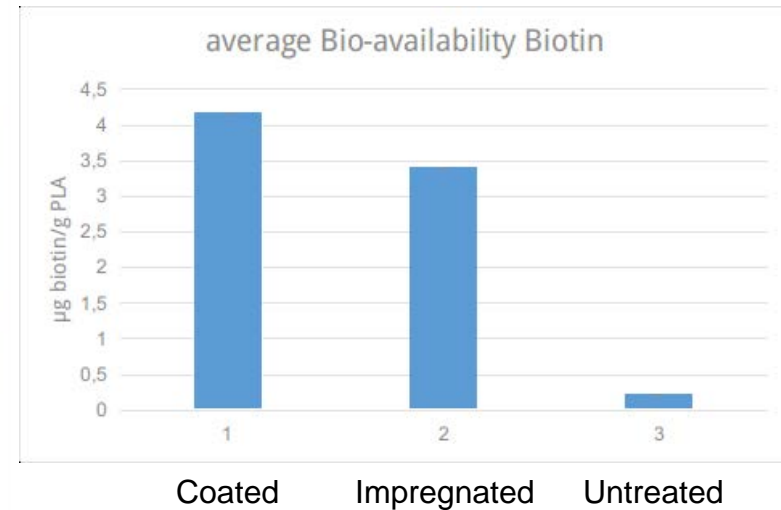
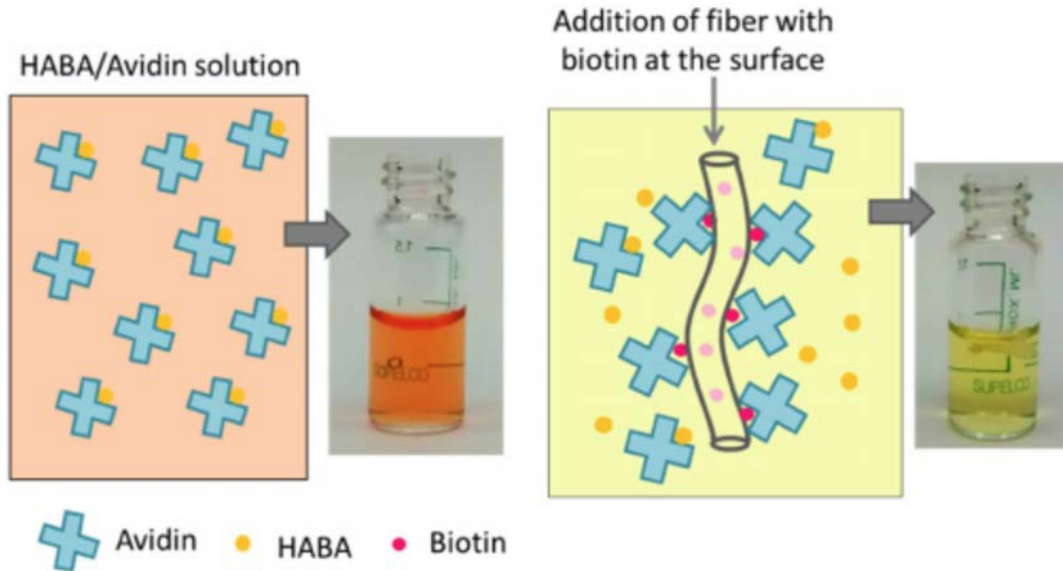
## Biotinylated PLA

- a) Chemical synthesis → too complicated
- b) Impregnated and remolten PLA:
  - Dissolve PLA + biotin in hot DMF
  - Crash out PLA by adding to EtOH
  - Wash with biotin saturated EtOH
  - Extrude new filament
- c) Coat with 'biotin paint':
  - DCM saturated with PLA, suspended biotin



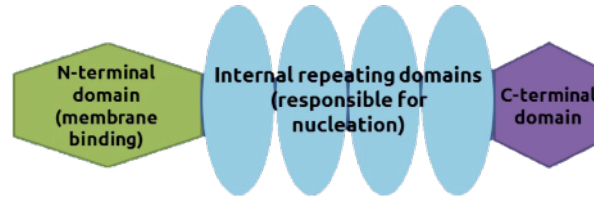
# WP 2: Filament

Test for addition of biotin: HABA/avidin assay

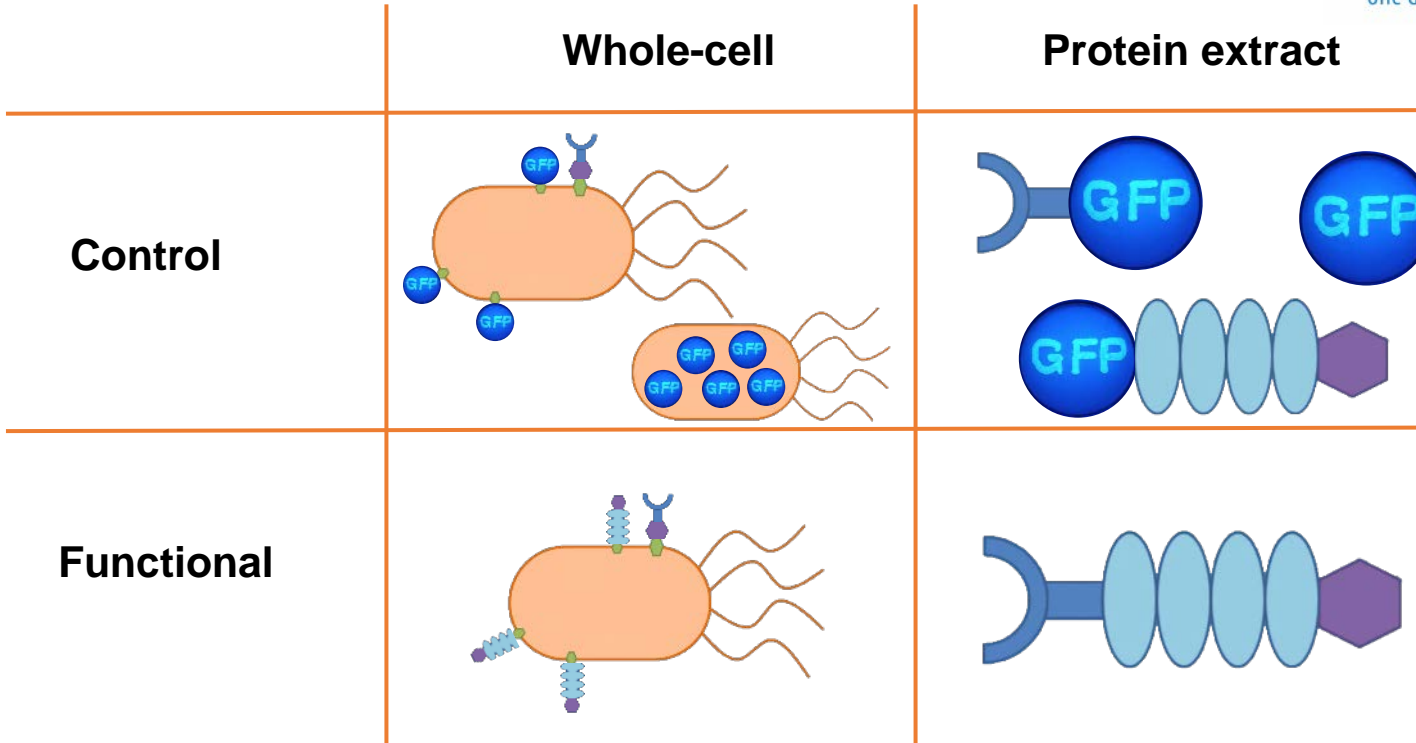




# WP 3: BioFunction



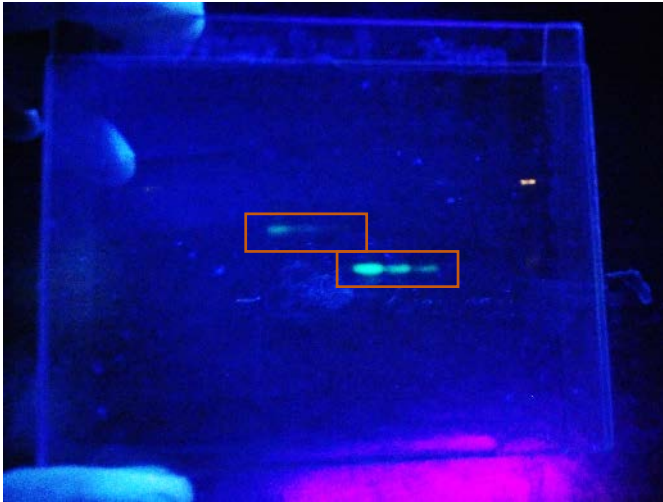
Setup constructs



# WP 3: BioFunction

The making of ...

A monomeric GFPuv (mGFPuv)



A GG-safe Ice Nucleating Protein





# WP 3: BioFunction

The making of ...

And a truncated Ice Nucleating Protein  
(INP-RC fusion proteins with GFP/streptavidin)



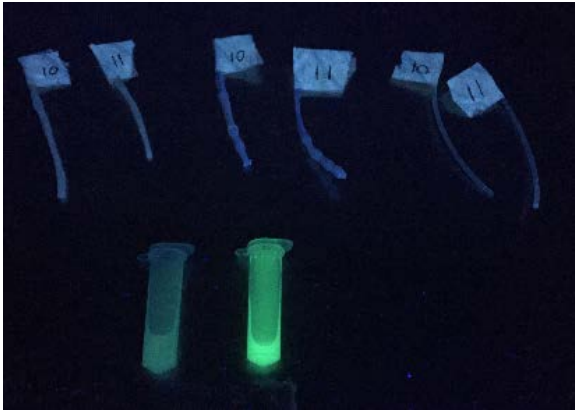
- |                    |                 |           |
|--------------------|-----------------|-----------|
| 1) inaZ(RC)-mSA2   | 3) mGFPuv-Strep | 5) mGFPuv |
| 2) inaZ(RC)-mGFPuv | 4) mGFPuv-mSA2  |           |

“Analogous ice forming movie”

# WP 4: Functional Assay

Does it stick?

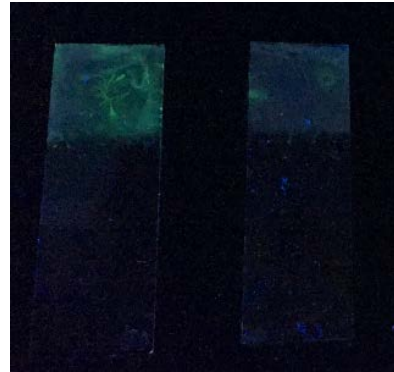
PLA filament+biotine



10: mGFPuv – mStrep

11: mGFPuv

PLA+biotine coated glass plate



Left: mGFPuv – mStrep ?

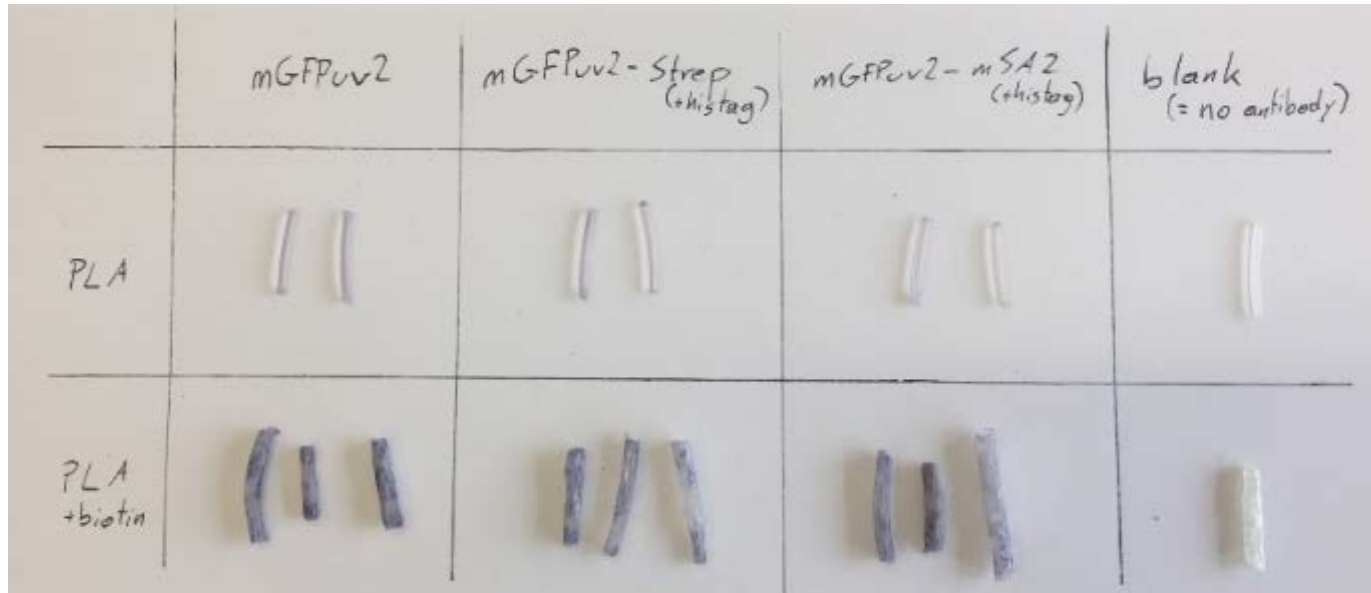
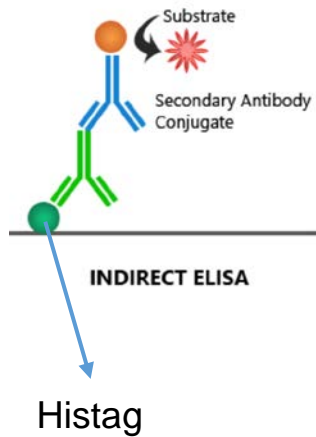
Right: a control..

Whole-cell approach no visible fluorescence  
(data not shown)

# WP 4: Functional Assay

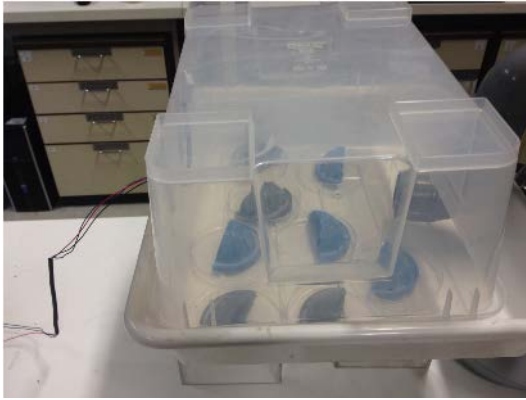
Does it stick?

... a more sensitive assay

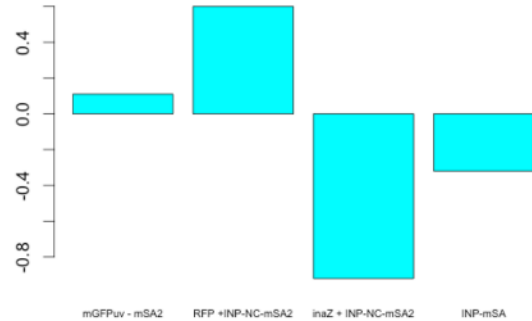


# WP 4: Functional Assay

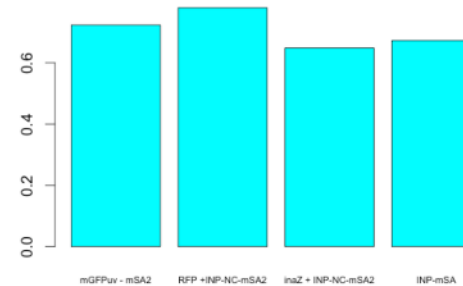
Does it make water?



Amount of water collected compared to control (gram)



Amount of water collected on coated microscopy glasses (gram)





Solving water shortage  
one drop at a time

Extraction of atmospheric water  
using an optimized 3D shape  
& engineered  
ice nucleating proteins



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@iGEM\_Ghent  
[http://2016.igem.org/Team:UGent\\_Belgium](http://2016.igem.org/Team:UGent_Belgium)



# dewpal



InBio.Be